



Micellar high performance liquid chromatographic determination of flunixin meglumine in bulk, pharmaceutical dosage forms, bovine liver and kidney



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ABSTRACT

A simple, sensitive and rapid liquid chromatographic method was developed and validated for the analysis of flunixin meglumine (flunixin-M) in bulk, pharmaceutical dosage forms, bovine liver and kidney. Analytical separation was performed in less than 4 min using a C₁₈ column with UV detection at 284 nm. A micellar solution composed of 0.15 M sodium dodecyl sulphate, 8% *n*-butanol and 0.3% triethylamine in 0.02 M phosphoric acid buffered at pH 7.0 was used as the mobile phase. The method was fully validated in accordance with the International Conference on Harmonization (ICH) guidelines. The limit of detection and the limit of quantitation were 0.02 and 0.06 µg mL⁻¹, respectively. The recoveries obtained were in range of 95.58–106.94% for bovine liver and kidney. High extraction efficiency was obtained without matrix interference in the extraction process and in the subsequent chromatographic determination. The method showed good repeatability, linearity and sensitivity according to the evaluation of the validation parameters.

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1. Introduction

Flunixin, 2-[[2-methyl-3-(trifluoro-methyl) phenyl] amino]-3-pyridine carboxylic acid (Fig. 1) [1], is usually found as its meglumine salt. Its actions are related to its ability to inhibit cyclooxygenase. It is used in horses for the alleviation of inflammation, pain associated with musculoskeletal disorders and visceral pain associated with colic. In cattle, it is indicated for the control of pyrexia associated with bovine respiratory diseases, endotoxemia and acute bovine mastitis [2]. Flunixin was the second leading violative residue reported in 2007, so the FDA Center for Veterinary Medicine (FDA-CVM) warned veterinarians to use flunixin in the proper and labeled manner. The FDA-CVM states that using a different route of administration for convenience is not adequate reason for extra label use, making most intramuscular or subcutaneous use of flunixin illegal. Given intravenous, the label withdrawal time is 4 days and the milk withdrawal is 36 h. Given intramuscular or subcutaneous the withdrawal time may be more in order of 40 days [3]. Flunixin has no Codex maximum residual limits (MRLs) approved for use at national level for food animals and

definitive MRLs have been established for use in veterinary medicinal products in the EC (European Commission) (Annex I of Regulation, No. 2377/90), in bovine liver 300 µg/kg and in the kidney 100 µg/kg [4].

Various methods have been reported for the determination of flunixin-M including electrochemical [5], gas chromatography [6–8], thin layer chromatography [9], spectrophotometric [10]. Few liquid chromatographic (LC) methods have been reported for its determination. It was determined in swine muscles and processed food using tandem mass spectrometric detection [11,12], also in horse urine, mutton muscle, pharmaceutical dosage forms and bovine plasma using UV detection [9,13–15].

Micellar liquid chromatography (MLC) allows complex matrices to be analyzed without the need of extraction and with direct injection of the samples [16]. Micelles tend to bind proteins competitively, thereby releasing protein-bound drugs and proteins, rather than precipitating into the column. Proteins are solubilized and washed harmlessly away, eluting with the solvent front. This means that costs and analysis times are cut considerably [17]. Micellar mobile phases usually need less quantity of organic modifier and generate less amount of toxic waste in comparison to aqueous–organic solvents, so that they are less toxic, non-inflammatory, biodegradable and relatively inexpensive [18]. MLC has proved to be a useful technique in the determination of diverse

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groups of compounds in several matrices [19–24], including food samples [25–28].

The aim of the present study was to develop simple, rapid, sensitive, selective and relatively inexpensive LC method for analysis of flunixin-M in bulk, dosage forms and in bovine liver and kidney with a simple and rapid sample preparation especially for the routine analysis.

2. Experimental

2.1. Materials

Pure flunixin-M sample was kindly supplied by Delta Pharma, Cairo, Egypt. Its purity was of 99.9% as stated by the supplier. Flunidyne injections, B.N. 0846/11, each mL is labeled to contain 83 mg flunixin-M equivalent to 50 mg flunixin, a product of Arab company for medical products, Egypt, purchased from local market. Bovine liver and kidney were purchased from the local market.

2.2. Reagents and chemicals

All reagents and solvents used were of HPLC grade. High purity water was used throughout the study.

Ortho-phosphoric acid (85%, w/v), 1-propanol and *n*-butanol were obtained from Sigma–Aldrich (Germany). Methanol and acetonitrile were obtained from Fisher Scientific (UK). Sodium dodecyl sulphate (SDS) was obtained from Oxford Laboratory, Mumbai (India). Triethylamine was obtained from SD-Fine-Chem. limited (India). Nylon filters and syringe filters were from Sartorius–Stedium (Goettingen, Germany).

2.3. Instrumentation

Chromatographic analysis was carried out using a Shimadzu Prominence HPLC system, (Shimadzu, Japan) with a LC-20 AD pump, DGU-20 A5 degasser, CBM-20A interface, and SPD-20A UV–Vis detector with 20 μ L injection loop. Centrifugation was carried out using a TDL-60 B Centrifuge (Anke, Taiwan). Ultrasonic bath used was BHA-180 T (Abbotta, USA) was used. Tissue homogenization was made using Tissue Master-125 with 7-mm stainless steel generator probe (Omni International, GA, USA). The pH was measured with Jenway pH meter, 4510, (Essex-UK). The mobile phase was filtered through Charles Austen Pumps Ltd. Filter, model-B100 SE (England, UK) using 0.45 μ m milli-pore filters (Gelman, Germany).

2.4. Chromatographic conditions

MLC was performed on Shim-Pack VP-ODS column (150 mm \times 4.6 mm i.d., 5 μ m particle size) Shimadzu, Japan using micellar mobile phase consisting of 0.15 M sodium dodecyl sulphate, 8% *n*-butanol and 0.3% triethylamine in 0.02 M ortho-phosphoric acid buffered at pH 7.0. The mobile phase was filtered and sonicated for 30 min before use. The flow rate was 1.0 mL/min and sample injection volumes were 20 μ L at room temperature (25 $^{\circ}$ C). The UV detector was operated at 284 nm.

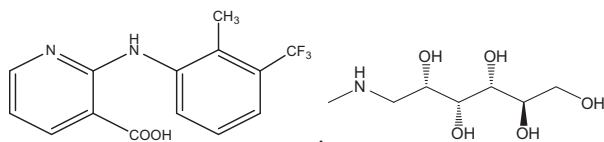


Fig. 1. Chemical structure of flunixin-M.

2.5. Standard solutions

Stock solution of 0.2 mg mL⁻¹ of flunixin-M was prepared by dissolving 10 mg flunixin-M in 50 mL of water then the solution was sonicated in an ultrasonic bath for 5 min. Working solutions were prepared by diluting the stock solution with the mobile phase. Stock solution was found to be stable for 5 days if stored in the refrigerator.

2.6. Preparation of calibration curves

Working solutions containing (0.1–2.0 μ g mL⁻¹) and (2.0–20 μ g mL⁻¹) of flunixin-M were prepared by serial dilutions of aliquots of the stock solution. Then, 20 μ L aliquots were injected (triplicate) and eluted with the mobile phase under the reported chromatographic conditions. The average peak areas were plotted versus the concentrations of the drug in μ g/mL. Alternatively, the corresponding regression equations were derived.

2.7. Application to injection

Five Flunidyne[®] injections were mixed and an aliquot of the mixed solution equivalent to 100 mg was transferred to a 100-mL volumetric flask and completed to volume with water to obtain a solution claimed to contain 1.0 mg mL⁻¹ flunixin-M. 10-mL of the above solution was diluted to 50 mL with water to obtain a drug solution claimed to contain 0.2 mg mL⁻¹. Solutions were analyzed following the details under “Preparation of calibration curves”.

2.8. Bovine liver and kidney samples preparation

2.5 g of the bovine liver or kidney was accurately weighed and spiked with aliquots of flunixin-M solution. The spiked samples were homogenized and completed to 25 mL of 0.15 M SDS solution of pH 7.0. The samples were homogenized at 5000 rpm for 5 min; then, the homogenate was sonicated for 15 min and then centrifuged at 3000 rpm for 5 min. The supernatant of the samples was filtered through 0.45- μ m membrane filters using vacuum pump. The filtrate was diluted with the mobile phase, filtered through syringe filter. Aliquots of 20 μ L were injected (triplicate) and eluted with the mobile phase under the above chromatographic conditions. The average peak area was plotted versus the concentration of flunixin-M in μ g mL⁻¹ to get the calibration curve.

3. Results and discussion

The proposed method permits the quantitation of flunixin-M in bulk, pharmaceutical dosage forms, bovine liver and kidney. The proposed method offers high sensitivity as low as 0.0196 μ g mL⁻¹ of flunixin-M could be detected accurately.

Different parameters affecting the chromatographic performance of flunixin-M were carefully studied in order to achieve the most suitable chromatographic system. The results of the optimization study can be summarized as follows:

3.1. Choice of appropriate detection wavelength

UV detection was set at different wavelengths depending on the absorbance properties of the drug (Fig. 2). It was found that, 284 nm is the optimal wavelength to maximize the sensitivity of determination of the drug.

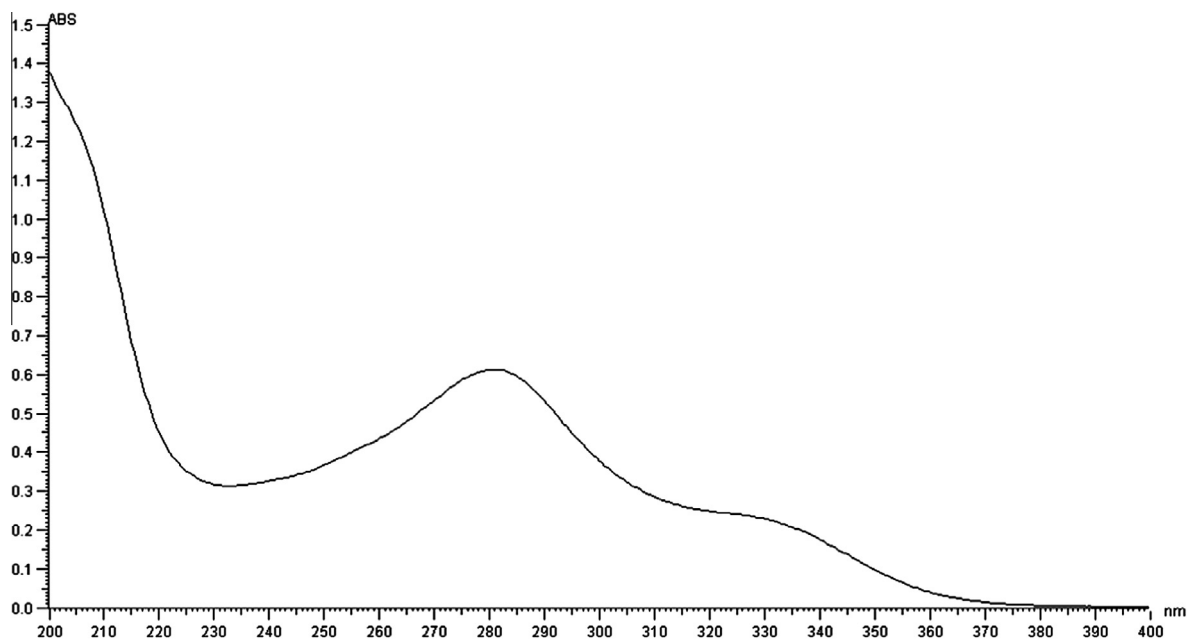


Fig. 2. Absorption spectrum of intact flunixin-M ($20 \mu\text{g mL}^{-1}$) in distilled water.

3.2. Choice of column

Two different columns were used for performance investigations, including: Shim-Pack VP-ODS C_{18} ($250 \text{ mm} \times 4.6 \text{ mm i.d.}$, $5 \mu\text{m}$ particle size) and Shim-Pack VP-ODS C_{18} ($150 \text{ mm} \times 4.6 \text{ mm i.d.}$, $5 \mu\text{m}$ particle size).

The experimental studies revealed that the second column was more suitable, since it produced well-resolved peaks in a reasonable time.

3.3. Mobile phase composition

To achieve the appropriate chromatographic conditions, the mobile phase composition was optimized to provide sufficient selectivity and sensitivity in a short separation time. The studied variables included; the pH of the mobile phase, concentration of

Table 1
Optimization of the chromatographic conditions for the determination of flunixin-M.

| Parameter | | No. of theoretical plates (N) | Capacity factor (K') | Tailing factor (t_r) |
|--|--------------|-----------------------------------|--------------------------|--------------------------|
| pH of the mobile phase | 4.0 | 1100 | 4.49 | 1.29 |
| | 4.5 | 1080 | 4.05 | 1.45 |
| | 6.0 | 1010 | 2.28 | 1.54 |
| | 6.5 | 1199 | 1.70 | 1.50 |
| | 7.0 | 1294 | 1.64 | 1.23 |
| | 7.5 | 1220 | 0.75 | 1.49 |
| Conc. of SDS (M) | 0.075 | 990 | 1.21 | 1.62 |
| | 0.1 | 1000 | 1.98 | 1.41 |
| | 0.125 | 1280 | 1.81 | 1.27 |
| | 0.15 | 1294 | 1.64 | 1.23 |
| | 0.175 | 1300 | 1.361 | 1.30 |
| Type of organic modifier of conc. 8% v/v | Butanol | 1294 | 1.61 | 1.23 |
| | Propanol | 870 | 1.20 | 1.27 |
| | Acetonitrile | 980 | 1.33 | 1.37 |
| | Methanol | 1100 | 1.72 | 2.2 |
| | Ethanol | 1013 | 1.98 | 1.82 |
| % of 1-butanol (% v/v) | 8% | 1294 | 2.0 | 1.23 |
| | 10% | 1300 | 1.54 | 1.20 |
| | 12% | 916 | 1.35 | 1.48 |
| Flow rate (mL/min) | 0.8 | 1005 | 1.71 | 1.52 |
| | 1.0 | 1294 | 1.64 | 1.23 |
| | 1.2 | 1300 | 1.61 | 1.59 |

SDS, the type and concentration of organic modifier and the flow rate. The results obtained are presented in Table 1.

3.4. Effect of pH

The effect of changing the pH of the mobile phase on the selectivity and retention time of flunixin-M was investigated using mobile phases of pH ranging from 4.0 to 7.5 with 0.15 M SDS concentration and 8% *n*-butanol. Table 1 shows that a pH of 7.0 was most appropriate, where it offers a good combination of peak symmetry and analysis time.

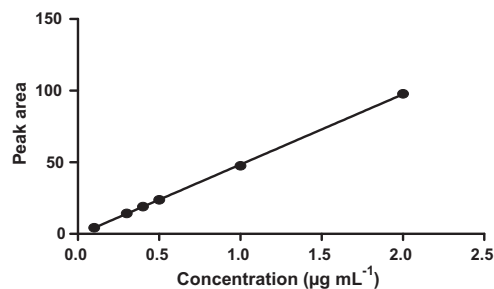


Fig. 3. Calibration graph for the HPLC determination of flunixin meglumine by the proposed method.

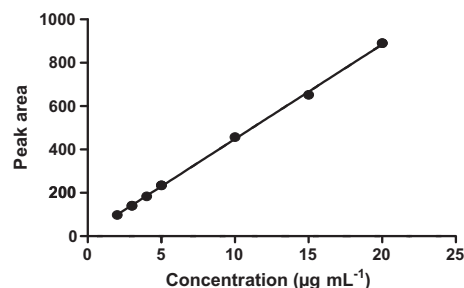


Fig. 4. Calibration graph for the HPLC determination of flunixin meglumine by the proposed method.

Table 2
Analytical performance data for the HPLC determination of flunixin-M.

| Parameter | Values | |
|--|---|--|
| | Working range (0.1–2.0 µg mL ⁻¹) | Working range (2.0–20 µg mL ⁻¹) |
| Intercept | -0.6604 | 11.39 |
| Slope | 49.06 | 43.66 |
| Correlation coefficient (<i>r</i>) | 0.9998 | 0.9993 |
| SD of residuals (<i>S_{y/x}</i>) | 0.4748 | 8.564 |
| S.D. of intercept (<i>S_a</i>) | 0.292 | 5.382 |
| S.D. of slope (<i>S_b</i>) | 0.3047 | 0.5102 |
| S.D. | 1.201 | 1.897 |
| % RSD ^a | 1.196 | 1.901 |
| (LOD) (µg mL ⁻¹) ^b | 0.02 | |
| (LOQ) (µg mL ⁻¹) ^c | 0.06 | |

^a Percentage relative standard deviation.

^b Limit of detection.

^c Limit of quantitation.

3.5. Concentration of SDS

SDS concentration was varied over the range of 0.075–0.175 M and containing 8% *n*-butanol and buffered at pH 7.0. Table 1 shows that 0.15 M SDS was the best, giving well-resolved peaks and the highest number of theoretical plates. Retention times increased when concentration of surfactant decreased.

Table 3
Assay results for the determination of flunixin-M in pure form by the proposed and comparison methods.

| Ranges | Proposed method | | | Comparison method | | |
|----------------|----------------------|----------------------|---------------|----------------------|----------------------|---------------|
| | Amount taken (µg/mL) | Amount found (µg/mL) | % Recovery | Amount taken (µg/mL) | Amount found (µg/mL) | % Recovery |
| 0.1–2.0 µg/mL | 0.1 | 0.102 | 101.19 | 5.0 | 5.212 | 104.20 |
| | 0.3 | 0.305 | 101.76 | 15.0 | 15.126 | 100.84 |
| | 0.4 | 0.403 | 100.81 | 30.0 | 30.212 | 100.71 |
| | 0.5 | 0.499 | 99.97 | | | |
| | 1.0 | 0.983 | 98.31 | | | |
| | 2.0 | 2.01 | 100.34 | | | |
| Mean% ± S.D. | | | 100.39 ± 1.20 | | | 101.91 ± 1.98 |
| <i>t</i> -test | 1.459 | | | (2.365) | | |
| <i>F</i> -test | 2.718 | | | (19.3) | | |
| 2.0–20 µg/mL | 2.0 | 1.979 | 98.95 | 5.0 | 5.212 | 104.20 |
| | 3.0 | 2.949 | 98.29 | 15.0 | 15.126 | 100.84 |
| | 4.0 | 3.939 | 98.47 | 30.0 | 30.212 | 100.71 |
| | 5.0 | 5.12 | 102.39 | | | |
| | 10.0 | 10.211 | 102.11 | | | |
| | 15.0 | 14.671 | 97.81 | | | |
| | 20.0 | 20.136 | 100.68 | | | |
| | Mean% ± S.D. | | | 99.81 ± 1.897 | | |
| <i>t</i> -test | 1.583 | | | (2.306) | | |
| <i>F</i> -test | 1.089 | | | (19.3) | | |

Each result is the average of three separate determinations.

The values between parentheses are the tabulated *t* and *F* values at *P* = 0.05.

Table 4
Accuracy and precision data for the determination of flunixin-M by the proposed method.

| Range | Amount taken (µg/mL) | Intraday ^a | | | Interday ^b | | |
|---------------|-------------------------|-----------------------------|------------------------|------------------|-----------------------------|------------------------|------------------|
| | | Amount found ± S.D. (µg/mL) | Accuracy (<i>R</i> %) | Precision (RSD%) | Amount found ± S.D. (µg/mL) | Accuracy (<i>R</i> %) | Precision (RSD%) |
| 0.1–2.0 µg/mL | 0.1 | 0.103 ± 0.002 | 103.00 | 1.942 | 0.102 ± 0.002 | 102.00 | 1.961 |
| | 0.4 | 0.405 ± 0.005 | 101.25 | 1.235 | 0.407 ± 0.007 | 101.75 | 1.72 |
| | 1.0 | 1.023 ± 0.011 | 102.30 | 1.075 | 0.997 ± 0.016 | 99.70 | 1.605 |
| 2.0–20 µg/mL | 2.0 | 2.012 ± 0.029 | 100.60 | 1.461 | 1.997 ± 0.037 | 99.85 | 1.843 |
| | 10.0 | 10.235 ± 0.175 | 102.35 | 1.71 | 9.95 ± 0.227 | 99.50 | 2.281 |
| | 20.0 | 20.096 ± 0.169 | 100.48 | 0.841 | 19.83 ± 0.292 | 99.15 | 1.473 |

Each result is the average of three separate determinations.

^a Within the day.

^b Three consecutive days.

3.6. Type of organic modifier

The effect of changing the type of organic modifier on the selectivity and retention time of flunixin-M was investigated using mobile phases containing 8% of either methanol, ethanol, 1-propanol, *n*-butanol, or acetonitrile and containing 0.15 M SDS and buffered at pH 7.0. Table 1 shows that 8% *n*-butanol was chosen as the best organic modifier.

3.7. Concentration of organic modifier

The concentration of *n*-butanol was varied over the range of 8–12%. Table 1 shows that there is no significant difference in the number of theoretical plates upon using either 8% or 10% *n*-butanol so 8% was chosen as it gave well-resolved peaks within a reasonable retention time. Hence, a small amount of *n*-butanol is added to accelerate and control the elution of the drug.

3.8. Flow rate

The effect of flow rate of the mobile phase on the retention of flunixin-M was studied over the range of 0.8–1.2 mL/min. Flow rate of 1 mL/min was optimal for good separation in a reasonable time (Table 1).

After optimization of these variables, best peak shape and lowest peak tailing were achieved with well-defined peaks and good sensitivity within a reasonable analytical run time.

3.9. Method validation

The validity of the proposed method was assessed by studying the following parameters in accordance to ICH Q2B recommendations [29]: linearity, LOD, LOQ, accuracy, precision, selectivity, sample solution stability, mobile phase stability and robustness.

3.10. Linearity

Under the above-described experimental conditions, linear relationships were established by plotting peak areas against the drug concentrations (Fig. 3 and 4). The concentration range was found to be (0.1–2.0 $\mu\text{g mL}^{-1}$) and (2.0–20 $\mu\text{g mL}^{-1}$). Linear regression analysis of the data gave the following equations:

$$P = -0.6604 + 49.06C \quad r = 0.9998 \quad (0.1\text{--}2.0 \mu\text{g/mL})$$

$$P = 11.39 + 43.66C \quad r = 0.9993 \quad (2.0\text{--}20 \mu\text{g/mL})$$

where C is the concentration of the drug in $\mu\text{g mL}^{-1}$ and P is the peak area.

The high value of the correlation coefficient (r value > 0.999) indicates good linearity of the calibration graph in both cases.

3.11. Limit of quantitation (LOQ) and limit of detection (LOD)

The limit of quantitation (LOQ) was determined by establishing the lowest concentration of the analyte that can be measured according to ICH Q2B recommendations [29] and below which the calibration graph is non-linear and was found to be 0.06 $\mu\text{g mL}^{-1}$. The limit of detection (LOD) was determined by establishing the minimum level at which the analyte can be reliably detected; it was found to be 0.02 $\mu\text{g mL}^{-1}$ as shown in Table 2.

3.12. Accuracy

To prove the accuracy of the proposed method, the results of the assay of flunixin-M in pure form by the proposed MLC method were compared with those of the comparison method. The comparison method is flunixin-M manufacturer method which depends on measuring UV-absorbance of the drug at 289 nm in ethanol.

Statistical analysis of the results obtained using Students' t -test and variance ratio F -test [30] revealed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively as illustrated in Table 3.

3.13. Precision

Intra-day precision was achieved by determination of three concentrations of flunixin-M on three successive times in the same day. Inter-day precision was performed as inter-day precision but on three successive days. Small values of % RSD revealed the precision of the proposed method. The results are illustrated in Table 4.

3.14. Selectivity

The selectivity of the proposed MLC method was established by its ability to determine flunixin-M in commercial injections solution without interference from any additives, Fig. 5b. Furthermore, to evaluate the specificity of the method to determine the cited drug in bovine liver and kidney, blank samples was prepared and injected under the recommended chromatographic conditions.

No interfering peaks were observed at the retention time of the drug, which proved the homogeneity and purity of the peak, Fig. 5c and d.

3.15. Sample solution stability and mobile phase stability

Evaluation of the stability of flunixin-M was achieved by quantification of the drug on five successive days and comparison to freshly prepared solution. No significant changes were observed, proving that it was stable for up to 5 days. The stability of the mobile phase was also checked, it was found to be stable for up to 3 days with no significant changes.

3.16. Robustness

To assess the robustness of the proposed MLC method, the chromatographic conditions were deliberately altered such as pH (7.0 ± 0.5), concentration of n -butanol ($8 \pm 0.5\%$, v/v) and concentration of SDS (0.15 ± 0.025 M). The efficiency of the separation of flunixin-M was not affected indicating the reliability of the proposed method. Therefore, the method is robust to the small changes in the experimental conditions.

4. Applications

4.1. Application of the proposed method to the determination of flunixin-M in its injection solution

The developed MLC method was applied successfully for the assay of flunixin-M in Flunidyne[®] injection solution as shown in

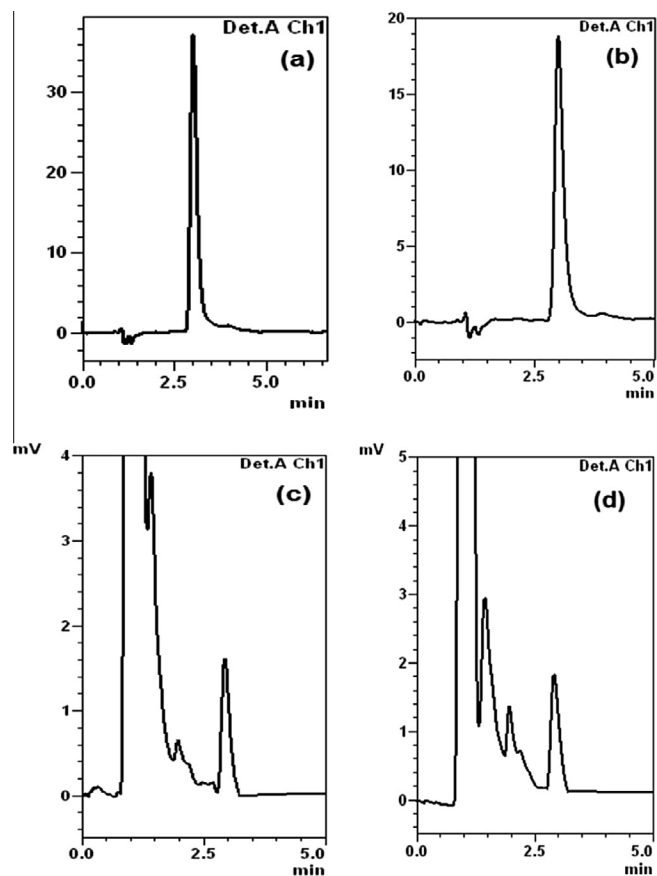


Fig. 5. Chromatograms showing (a) flunixin-M standard (10 $\mu\text{g/mL}$); (b) flunixin-M (5 $\mu\text{g/mL}$) in its dosage form; (c) flunixin-M in liver sample (0.5 $\mu\text{g/mL}$); (d) flunixin-M in kidney sample (0.5 $\mu\text{g/mL}$).

Table 5
Assay results for the determination of flunixin-M in injection by the proposed and comparison methods.

| Parameters | Proposed method | | | Comparison method | | |
|-----------------|-----------------------------------|-----------------------------------|--------------------|-----------------------------------|-----------------------------------|--------------------|
| | Amount taken ($\mu\text{g/mL}$) | Amount found ($\mu\text{g/mL}$) | % Recovery | Amount taken ($\mu\text{g/mL}$) | Amount found ($\mu\text{g/mL}$) | % Recovery |
| Data | 2.0 | 1.956 | 97.81 | 5.0 | 5.04 | 100.79 |
| | 3.0 | 3.025 | 100.85 | 15.0 | 14.917 | 99.45 |
| | 4.0 | 4.08 | 101.99 | 30.0 | 30.009 | 100.03 |
| | 5.0 | 5.027 | 100.55 | | | |
| | 10.0 | 10.097 | 100.97 | | | |
| | 15.0 | 14.538 | 96.77 | | | |
| | 20.0 | 20.263 | 101.38 | | | |
| Mean \pm S.D. | | | 100.04 \pm 1.961 | | | 100.09 \pm 0.677 |
| t-test | 0.04 | | | (2.306) | | |
| F-test | 8.397 | | | (19.3) | | |

Each result is the average of three separate determinations.
The values between parentheses are the tabulated *t* and *F* values at *P* = 0.05.

Table 6
Assay results for the determination of flunixin-M in bovine liver and kidney by the proposed method.

| Method | Bovine liver | | | Bovine kidney | | |
|--------|-----------------------------------|-----------------------------------|------------|-----------------------------------|-----------------------------------|------------|
| | Amount taken ($\mu\text{g/mL}$) | Amount found ($\mu\text{g/mL}$) | % Recovery | Amount taken ($\mu\text{g/mL}$) | Amount found ($\mu\text{g/mL}$) | % Recovery |
| Data | 0.2 | 0.214 | 100.84 | 0.2 | 0.211 | 105.62 |
| | 0.5 | 0.478 | 95.58 | 0.5 | 0.482 | 96.43 |
| | 1.0 | 1.008 | 106.94 | 1.0 | 1.007 | 100.62 |
| Mean | | | 101.12 | | | 100.91 |
| S.D. | | | 5.685 | | | 4.601 |
| % RSD | | | 5.622 | | | 4.56 |

Each result is the average of three separate determinations.

Fig. 5. The results obtained were statistically compared with those of the comparison method using *t*-test and *F*-test. The results show that there were no significant differences between the developed and comparison method regarding accuracy and precision, respectively as illustrated in **Table 5**.

4.2. Application of the proposed method to the determination of flunixin-M in bovine liver and kidney

The applicability of the procedure developed to determine flunixin-M was tested by analyzing the drug in bovine liver and kidney. All samples were bought at a local Supermarket. **Table 6** shows the results of the analysis of flunixin-M determined in all samples after homogenization with micellar solution, sonication, centrifugation and filtration. The data obtained (**Table 6**) show satisfactory recoveries for flunixin-M in all samples, and the results fall in the range of 95.58–106.94%. **Fig. 5** shows the chromatograms obtained from the spiked samples of flunixin-M analyzed with the optimum mobile phase.

5. Conclusion

The proposed method is useful for food quality testing and control areas to determine the content of flunixin-M in bovine liver and kidney samples. One advantage of this procedure is possibility of injecting the samples directly into the chromatographic system without previous treatment other than homogenization, dilution and filtration, thus avoiding tedious extraction from matrices. Validation according to ICH regulations provides satisfactory results in terms of sensitivity, linearity, accuracy and recoveries. It is noteworthy that the use of micellar mobile phase endows the procedure advantages such as non-toxicity, non inflammability, biodegradability, and low cost.

Conflict of interest

There are no conflicts of interest.

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